SURFACE MODIFICATION FOR BIOCOMPATIBILITY

First Quarterly Report
Covering Period December 15, 1994 to March 31, 1995
Contract No: N01-NS-5-2321

James J. Hickman, PhD
Science Applications International Corporation
Life Sciences Operation
1710 Goodridge Drive, MS 188
McLean, VA 22102

Submitted to:

Neural Prosthesis Program
Division of Fundamental Neurosciences
National Institute of Neurological Disorders and Stroke
National Institutes of Health
Federal Building, Room 9C02
Bethesda, MD 20892-9170

TABLE OF CONTENTS

	Page
PROJECT SUMMARY	1
OBJECTIVES	1
BACKGROUND	2
RESULTS	4
NEXT QUARTER OBJECTIVES	6

FIGURES

Figure 1 High resolution XPS scans of the N1S region of PEDA (top), MAP (middle), and DETA (bottom).

Figure 2 High resolution XPS scans of the N1S region of EDA (top), APTS (middle), and PL (bottom)

TABLES

Table 1 A qualitative assessment of both cortical cell survival and neurite outgrowth on three different surfaces under different conditions after 24 hrs. *in vitro*.

Table 2 Atomic percents of surface composition before and after cell culture.

PROJECT SUMMARY

The aim of this work is to create surfaces on implantable silicon microstructures for the purpose of controlling the interaction of neurons, glia, and related cells and their protein products with the microstructure. This first quarter was devoted to establishing the serum-free *in vitro* culture protocol and to begin preliminary surface screening experiments. The longest culture times achieved to date are 11-14 days. This will increase to 3-4 weeks with additional steps that have been successful for hippocampal cultures. Five artificial surfaces to date have been screened for E19 or E17 cortical cell response. XPS analysis has been done on each surface and the results will be included in a multivariant analysis treatment of all the variables. XPS protocols for analyzing the surfaces after culture have been established and preliminary data are being collected. We have added a cell biologist with extensive experience in cortical cell culture to work on the project. We have begun a collaboration with the Huntington Medical Research Institute to provide them surfaces for implantation studies of biocompatibility in rat CNS.

OBJECTIVES

Overall project objectives:

a) Selecting candidate surfaces that are likely to enhance the microscopic mechanical stabilization of a microstructure implanted within the central nervous system;

b) Selecting candidate organic surfaces that are likely to enhance the close approximation of neurons or neuronal processes to specific regions of implanted silicon microstructures;

c) Developing or adapting available methods to bond the selected organic molecules to a silicon dioxide surface like the surface of a micromachined electrode (Tanghe and Wise: A 16-channel CMOS neural stimulating array.

IEEE Trans. Sol State Circuits 27:69-75, 1992) and to chemically characterize these surfaces before and after protein adsorption.

1. The attachment method shall be stable in saline at 37°C for at least 3 months;

2. To use silane coupling as the method of attachment;

3. To use the silanes to control the spatial extent (i.e., the pattern) of the deposited surface.

d) Developing a cell culture or other suitable model of mammalian cortex and investigate the growth and adhesion of neurons, glia, micro-glia, and other cells present in the nervous system on substrates coated with the selected surfaces;

e) Cooperating with other investigators in the Neural Prosthesis Program by coating microelectrodes (estimated 50 over the contract period) with the most promising materials for *in vivo* evaluation as directed by the NINDS Project Officer.

First Quarter Objectives:

- Establish cortical cell culture protocols
- Begin *in vitro* cell culture screening experiments with rat cortical neurons on artificial surfaces
- Apply XPS analysis to the surfaces before and after culture
- Hire personnel with relevant experience to participate on project
- Begin collaborations with other members of the Neural Prosthesis Program

BACKGROUND

Biomaterials that penetrate into the central nervous system as the microscopic electrode shafts of neural prostheses interact with neural and other tissues on a

cellular and molecular level. In order to achieve tight coupling between these implanted microelectrodes and the target neural substrate, this interaction must be understood and controlled. Controlling the interaction requires an understanding of how cells, including neurons and glia, and extracellular proteins respond to the surface chemistry and any leachable substances of implanted biomaterials. This contract supported research will study these interactions with a long-term goal of rationally designing microeletrode surfaces to promote specific tissue interactions.

Presently, available clinical neural prosthetic implants typically use stimulus levels that excite volumes of neural tissue ranging from cubic millimeters to cubic centimeters around the electrode. Because of the large stimulus intensities required, precise control of the response of neurons within the first few cell layers of an implanted electrode has not been necessary. Recent developments in the areas of micromachining and fabrication of silicon integrated circuit microelectrodes have introduced the possibility of controlled stimulation of smaller volumes of neural tissue--on the order of one thousand to one hundred thousand times smaller than those used today.

The efficiency of these microelectrodes depends on the micro environment around stimulating sites. The surface of the microelectrodes and the proteins that adsorb to this surface have a major impact on the way in which different cell populations react to the implant. Neural growth cones are sent out from many neurons around a microelectrode following implantation. With appropriate surfaces it may be possible to get selected neurons to send processes directly to the microelectrodes. Glia and other cells also respond to an implanted electrode. With appropriate surfaces it may be possible to get cell adhesion and anchoring of some areas of the implant structure while leaving other areas with minimal response from glial cells. This study will investigate cellular and molecular responses to specific surface modifications of silicon microelectrodes.

RESULTS

Cell Culture

We are in the process of setting up the facility at George Mason University for cortical work. This was delayed due to some procedural details at the university that have been rectified. We have established a rudimentary serum-free culture system for the cortical cells to more closely approximate the composition of cerebral spinal fluid (CSF). We have started screening the artificial surfaces for neuronal growth and survival. The preliminary cell culture results are listed in Table 1. A detailed morphological analysis will be performed. The initial results indicate a wide variety of responses to the surfaces. Preliminary longevity was 10-14 days. Large scale screening will not be done until 3-4 week survival is achieved with cortical cells. We do not believe this will be a problem as we have successfully established this timeframe with hippocampal cells.

XPS Analysis

XPS is necessary for this program in the same way that an NMR spectrometer is necessary for conducting an organic synthesis program. Since we are synthesizing surfaces and modifying their properties, we will need to assay the result of the surface before (starting material) and after (reaction product) modification. This is analogous to examining a procedure by NMR. One would not think to run an organic synthetic reaction with only an occasional examination of the actual product; in the same sense, it is crucial for us to examine the product in our surface modification experiments.

XPS analysis was performed on the surfaces listed above for the cortical experiments. Representative XPS spectra for the nitrogen region are shown in Figures 1 and 2. Preliminary calculations of surface composition for all elements are

included in Table 2. This data, after further workup, will be incorporated in a multivariant analysis of factors affecting cortical growth and survival. XPS analysis of substrates following culture have just been completed for the earliest experiments. These have not been fully analyzed so the results are not included in this report. They will be fully documented in the second quarterly report.

Personnel

Margaret Coulombe, B.S., M.S., Biological Sciences

Ms. Coulombe joined SAIC as a Staff Cell Biologist with responsibility for culturing explanted neuronal elements and maintaining neuronal cell lines as well as assisting in the growth and maintenance of cells on the surface of the electrodes.

David R. Jung, PhD, Physics

Dr. Jung has joined SAIC as a Staff Surface Chemist. He has studied the chemical interactions of metal overlays on self-assembled monolayers (SAMs) using FTIR and XPS techniques for the past 4 years. As a member of the Life Sciences Operation, he manages the XPS service center, prepares SAM-modified substrates for cell culture, and conducts XPS surface analysis of samples before and after cell culture. Dr. Jung is responsible for modifying the surface of electrodes as well as XPS characterization of the surfaces.

Collaborations

We have established a collaboration with Dr. Agnews' group at the Huntington Medical Research Institute. We have coated silastic tubing with an artificial layer that has been shown to reduce protein adsorption. These have been implanted in rat spinal cord canals for 6 weeks. When removed, histological and surface analysis

will be done to show the efficacy of the treatment to reduce unwanted protein absorption and other resultant tissue buildup.

NEXT QUARTER OBJECTIVES

- Establish cortical cell culture conditions for 3-4 week survival
- Continue screening of surfaces for cortical cell survival
- Continue surface analysis of artificial surface both before and after cell culture
- Begin surface stability experiments in saline at 37°C
- Begin screening non-ideal artificial surfaces for glial cultures

TABLE 1

A qualitative assessment of both hippocampal cell survival and neurite outgrowth on three different surfaces under different conditions after 24 hrs. *in vitro*.

- – poor
+ to +++ = fair to very good.

Silane (Chemical Name)	Abbreviation	Survival* of Cortical Neurons Days *in vitro*			
		1	4	7	10,11
Trimethoxysilylpropyldiethylenetriamine	DETA	+++	++	++	+
N-Methylaminopropyltrimethoxysilane	MAP	++	++	+	+
(Aminoethylaminomethyl) Phenethyltrimethoxysilane	PEDA	++	++	+	+/-
3-Aminopropyltrimethoxysilane	APTS	++	++	+	+/-
N-(2-Aminoethyl)-3-Aminopropyltrimethoxysilane	EDA*	++	+++	++	+/-
Poly-D-Lysine	P-D-L*	+++	++	++	+

Cell Culture Methods:

Cells were dissociated mechanically and cultured at a density of 330 cells per mm^2 and maintained for 14+ days in MEM-N3.

TABLE 2

Atomic percents of surface composition before and after cell culture

	Si	C	N	O
substrate				
before culture				
APTS	29.1	12.6	2.8	55.5
DETA	28.9	12.2	3.2	55.5
MAP	27.7	9.3	2.8	60.2
PEDA	28.6	15.8	3.1	52.3
after culture				
PL	9.0	53.8	10.6	26.6
APTS	11.2	44.9	11.4	32.6
DETA	9.7	51.8	10.5	27.7
MAP	13.6	43.4	9.7	33.3
PEDA	9.9	51.8	11.1	27.2

Figure 1

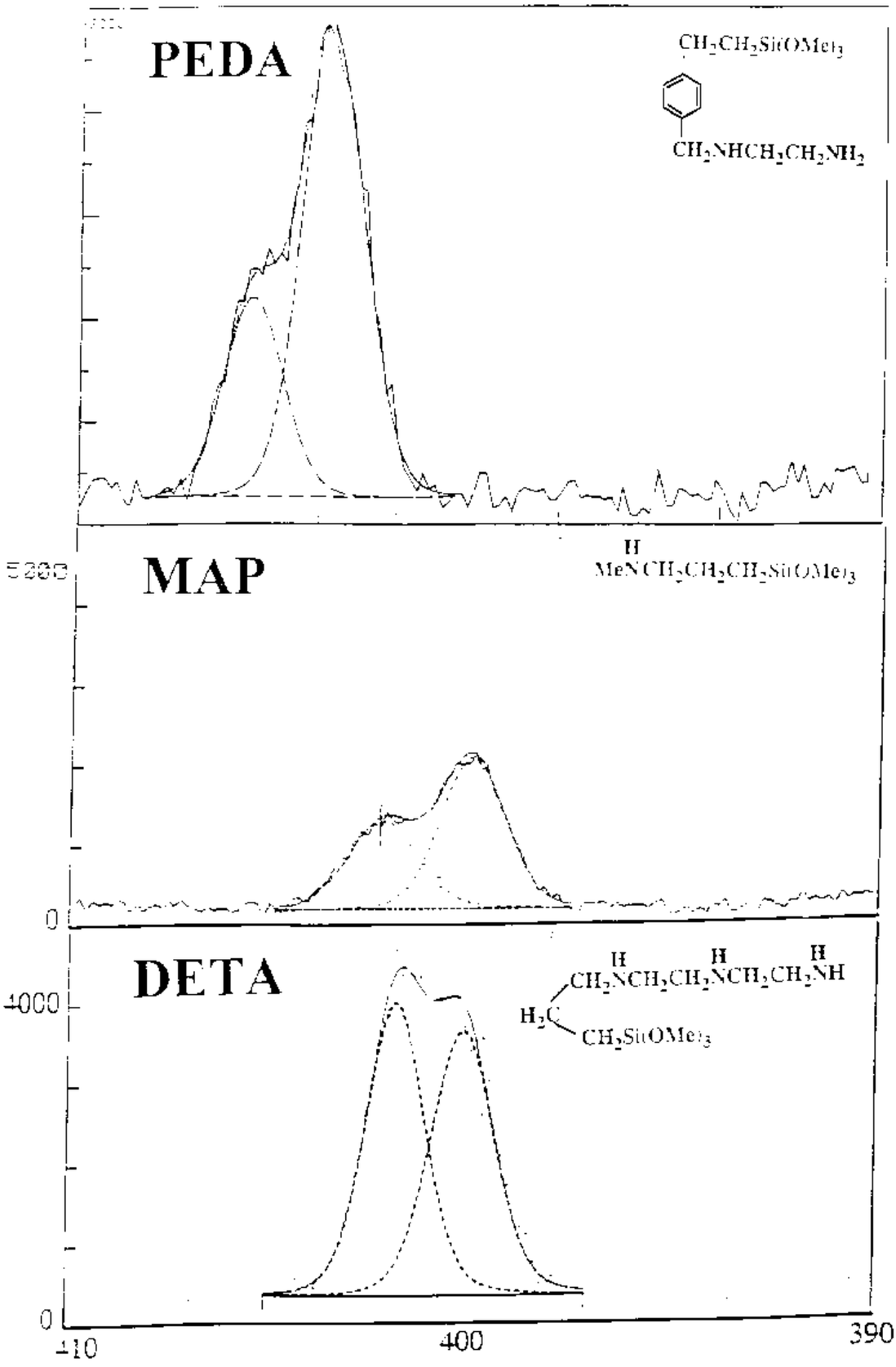
PEDA
$CH_2CH_2Si(OMe)_3$
$CH_2NHCH_2CH_2NH_2$
MAP
H
$MeNCH_2CH_2CH_2Si(OMe)_3$
0
DETA
4000
H H H
$CH_2NCH_2CH_2NCH_2CH_2NH$
H_2C
$CH_2Si(OMe)_3$
0
410
400
390
Binding Energy (eV)

Figure 2